Studies on Bio sustainability of PHB Production Using Soya Protein from Halophilic Microorganisms

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1. Introduction:

Plastic is a versatile material, and its production is increasing. Every year, millions of tons of non-biodegradable plastics end up in our natural environment, wreaking havoc on our health, wildlife, terrestrial, and marine habitats. As a result, polyhydroxybutyrate (PHBs) are biodegradable and biocompatible polymers that have been promoted as a replacement for traditional oil-based plastics. Since the Industrial Revolution, the increasing use of petroleum-based plastics has caused long-term problems due to depletion of oil sources, accumulation of plastic waste in nature, and rising oil prices.Poly-3-hydroxybutyrate (PHB) is a linear polyester of D (-)-3-hydroxybutyric acid discovered in bacteria by Maurice Lemoigne in 1926 as a constituent of the bacterium *Bacillus megaterium*. Under conditions of nutrient limitation other than the carbon source, it is accumulated in intracellular granules by a wide range of Gram-positive and Gram-negative organisms. The polymer, which serves as a carbon and energy reserve. The most significant advantages of using bioplastics are that they can be produced from renewable resources and are environmentally friendly. Polyhydroxyalkanoates, the most common type of bioplastic produced microbiologically, are the most common type of bioplastic (PHBs). With over 100 different monomer constituents and linear-structured polymers, this is a versatile class of polymers (Manel Ben Abdallah*et.al.* 2020).

The most common PHA form produced by microorganisms is poly-3-hydroxybutyrate (PHB), which is formed from 3-hydroxybutyric acid monomers via the polymerization reaction of three enzymes: 3-ketothiolase, acetoacetyl-CoAreductase, and PHA synthase. Under stress conditions, microorganisms synthesise polymers from acetyl-CoA, and 3-ketothiolase condenses two acetyl-CoAs to form acetoacetyl-CoA. Acetoacetyl reductase converts acetoacetyl-CoA to (R) -3-hydroxybutynl-CoA and PHA synthase polymerize (R) -3-hydroxybutyryl-CoA to PHB Excess carbon and the absence of an essential nutrient such as oxygen, phosphorus, nitrogen, or sulphur, or the absence of a trace element such as magnesium, calcium, or ferrous iron, are the stress conditions that cause microorganisms to produce these polymers. PHB is a stiff, highly crystalline, and relatively brittle thermoplastic. While PHB has a melting point of 175°C, its degradation temperature is 185°C, making injection molding difficult. Although the mechanical properties of PHB, such as Young's modulus (3.5 GPa) and tensile strength (MPa), are similar to those of polypropylene, the elongation to break of PHB (6 percent) is significantly lower than

that of polypropylene (400). In the developed PHB copolymer P(3HB-co-3HV), as the fraction of the 3-hydroxyvalerate unit increases, the impact strength and elongation to break increase; however, as a result, the polymer becomes stronger and more flexible. The injection moldings of the copolymer occur without thermal degradation due to the decrease in melting point**(**Benjamin Ragan Waters2007).

PHB has some beneficial properties, including moisture resistance, water insolubility, optical purity, and good oxygen impermeability. These characteristics distinguish them from other biodegradable plastics, which are either water-soluble or moisture sensitive. PHB or P(HBHV) biodegradation occurs when microorganism's colonies the polymer's surface and secrete enzymes that degrade the polymer. The most important characteristics of PHB and other polyhydroxyalkanoates are that they do not degrade under normal storage conditions and are stable in air. Cellulosic esters, starch derivatives, PHB, polylactic acids (PLA), and polycaprolactone are currently the most important types of bioplastics suggested by markets (PCL). These polymers are used in a variety of applications, including pharmaceutics, biomedical products, packaging, and agriculture. Bioplastics are gaining popularity around the world. In 2010, the total global production of bioplastics was 724,500 t/year. In 2011, approximately 1,000,000 t were produced, and it is expected that global bioplastic production will reach 1,709,700t/year by 2015. However, when compared to conventional plastics derived from petroleum, global consumption levels of bioplastics are low (Ivo Diego de Lima Silva *et.al* 2020).

Many halophilic microorganisms use polyhydroxybutyrate (PHB) as an intracellular carbon storage mechanism. PHA is produced by various microorganisms and is easily degraded by them.

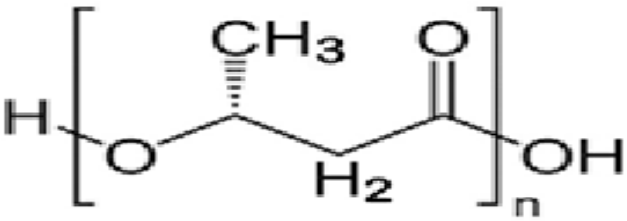

Fig Structure of PHB (Lara L. *et.al* 1999).

PHB Metabolic Pathway

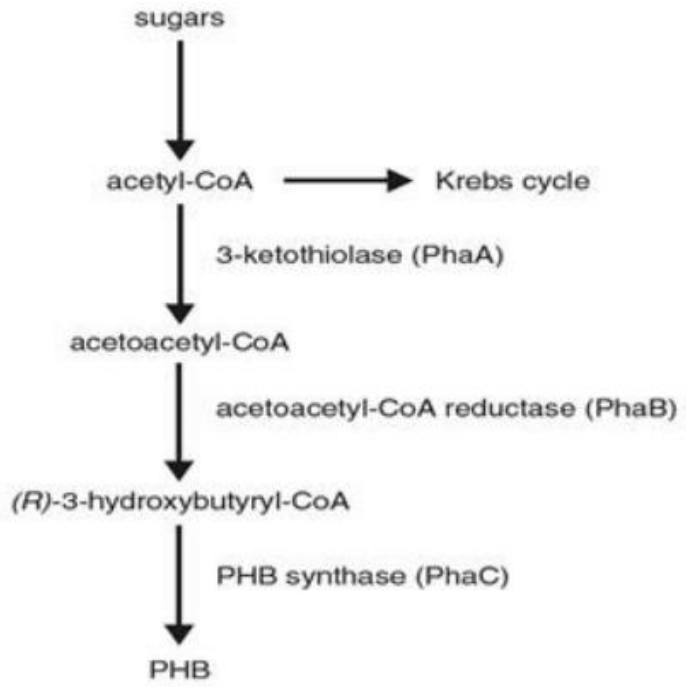

Fig: Metabolic Pathway of PHB (Mohsen Zarei2013).

Properties of PHB

- PHB is relatively resistant to hydrolytic degradation and is insoluble in water.
- A currently available biodegradable plastic differs from PHB in terms of water solubility or moisture sensitivity.
- PHB is an excellent candidate for an oxygen permeable polymer.
- PHB has low acid and base resistance but high ultraviolet resistance.
- PHB has a good solubility profile in chloroform and other chlorinated hydrocarbons. Biocompatibility is potential evidence for its use in biomedical applications and pharmaceutical formulation trends.
- The melting point is 175°C, and the glass transition temperature is 2°C. PHB has tensile strength at 40 MPa that is comparable to polypropylene.
- PHB demonstrates anaerobic biodegradation because it sinks in water, whereas polypropylene floats.
- Non-toxic.

- It's less sticky. (Akshay S.Jirage*et.al* 2013).

PHAs are produced by a wide range of bacteria and archaea from various carbon sources and are used as intracellular storage compounds in order to survive in unbalanced environments. Halophiles are organisms represented by archaea, bacteria, and eukarya for which the main characteristic is their salinity requirement, halophilic "salt-loving". Halophilic microorganisms constitute the natural microbial communities of hypersaline ecosystems, which are widely distributed around the world. They require sodium ions for their growth and metabolism. Thus, based on the NaCl optimal requirement for growth the halophiles are classified in three different categories: slight (1–3%); moderate (3–15%); and extreme (15–30%) (Paulina Corral 2020).

Extremely Halophilic archaea that live in hypersaline environments with high salt concentrations are preferred for a variety of potential applications, including polyhydroxyalkanoate synthesis. Poly3-hydroxybutyrate (PHB) was the first bioplastic discovered in a Dead Sea Halobacterium species described in 1972 There have been few studies into the production of PHB and other PHAs. Some Haloarchaeal strains in the genera *Haloferax, Haloarcula, Haloquadratum, Halorubrum, Halobiforma, Halorhabdus, Halalkalicoccus, Halobacterium, Natrianema, Halostagnicola, Natrinema, Natronobacterium, Natronorubrum, Haloterrigena, Halococcus.* Most haloarchaeal cells synthesis PHB or poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) at levels ranging from 0.8 percent to 22.9 percent (w/w) of cell dry weight, according to detailed analyses. *Haloferax mediterranei* is currently the best PHA-producing strain of extremely Halophilic archaea, producing PHB-co-PHV up to 60% of its dry cell weight with starch or glucose as a carbon source under phosphate limitation.Identify PHB-producing isolates using a specific PHB staining method, both phenotypically and genetically, and determine PHB yields using various media formulations. We would want to see how different media formulations affected production yields and to learn more about how environmental factors affect PHB production (Sherif M. El-Kadi *et.al* 2021).

Soybean contains approximately 40% protein and 20% oil on an average dry matter base. By removing oil at lower temperatures, soy protein isolate (SPI) is obtained, and is widely used in the food industry. Soy proteins are globular proteins with excellent film-forming properties along with biodegradability and low cost in nature. The major drawbacks such as low

mechanical, water, and thermal barrier properties associated with this polymer have restricted their application in packaging industries (Tian Tian *et.al* 2019).

Biopolymers made of PHB and biopolymers made of soya protein have no strengthening properties and are not resistant to high temperatures, moisture and acids. As a result, when soya protein is added to PHB, it undergoes a modification that may improves its straightness, temperature resistance, acid resistance, yield and other chemical and physical properties. The main goal of these projects is to determine which ligands and couplers were used to couple these soya proteins to PHB.

2. Review of Literature:

2.1 Analysis of Water

Water is one of the most important natural resources. It is essential for all living organisms, the majority of ecological systems, human health, food production, and economic development. The safety of drinking water is critical to one's health. Various contaminants, including chemical and microbiological contaminants, have an impact on the safety of drinking water. Such contaminants are hazardous to one's health. The quality of the drinking water degrades as a result of these contaminants. Because contaminated water can cause a variety of diseases in humans, the quality of the water must be tested for both chemical and microbial contaminants. During the study, the maximum number of physical and chemical parameters were found to be within the desirable limit, as suggestedby WHO (1971) and BIS (1991)(Devangee shukla *et.al* 2013).

Temperature, pH, TDS (Total Dissolved Solid), Electrical conductivity, DO (Dissolved oxygen), Free CO2, Carbonates (CO3) and Bicarbonates, Hardness, Chlorides, Salinity, Calcium and Magnesium Hardness were all investigated during the study period. The physical configuration of the crater, as well as its relative geographic and ecological isolation, influence Limnological status in a unique way. The ecosystem is highlighted as an ecological wonder due to its unusual and climatic isolation. The current study examines physicochemical parameters in order to determine the level of pollution and the state of eutrophication in Lonar Crater Lake. The study of hydrological status reveals that salinity varies during the rainy season and summer, indicating that the lake is approaching Eutrophication. Lonar Lake, Physico-chemical Parameter are the key words(Tandale M. R. and D.S. Dabhade2014).

2.2 Screening of PHB

Microorganisms (such as *Ralstonia eutrophus*, *Methylobacterium rhodesianum*, or *Bacillus megaterium*) produce PHB in response to physiological stress, primarily nutrient scarcity. Because HM medium contains a high salt concentration, it was used in this study to isolate PHB producer halophiles. In total, 21 isolates were discovered in four different marine samples collected from various locations in the Valsad district. M medium was used for secondary screening, and HM medium containing Nile blue A dye was used for final screening. Following the final screening, a total of seven different isolates were chosen as PHB producers and studied further. Fed-batch fermentation in flask was used to produce PHB from a single isolated strain. PHB was extracted in its crude form.Ethyle acetate extract was used to extract crude PHB, which was then characterised using an FTIR study (Nidihi patel*et.al* 2017).

Polyhydroxybutyrate (PHB) is the most extensively researched biodegradable plastic that, unlike petroleum-based plastics, does not release any toxins or residues into the environment. A total of sixteen isolates were collected and purified in this study to screen PHB accumulating microorganisms from marine sponges. To confirm the production of PHB, isolated strains were stained with Nile blue and observed under a Leica LSCM. PHB accumulation was found in a yellow pigmented AB8a isolate from Oceanopia arenosa, which was subjected to morphological, biochemical, and phylogenetic analysis. (Deepa Aryaraj , *et.al*2021).

2.3 Biochemical analysis

Extremophile organisms, such as halophiles, thrive in environments with extremely high salt concentrations. The word "salt-loving" is derived from Greek. While the term is most commonly associated with halophiles belonging to the Archaeadomain, it also applies to bacteria and some eukaryota, such as the alga*Dunaliellasalina*. By the severity of their halotolerance, halophiles are classified as mild, moderate, or extreme. Enrichment culture is used to isolate halophiles, and halophilic medium is used as a selective media. Each isolate is subjected to biochemical testing. S8 isolate was found to be an extreme halophile, S5 and S1 isolates were

moderate halophiles, and other isolates were unable to adapt to high salt concentrations. (Mohsin Azhar *et al*, 2014).

2.4Identification of halophilic bacterial species

Accurate identification of bacterial isolates is critical. Phenotypic methods are time-consuming and may fail to identify some bacteria, such as Gram-positive rods, completely or in some clinical situations. 16S rRNA sequencing is a relatively new method of identification that can be a good substitute. The purpose of this study is to see if this method can be used to identify a variety of bacteria in a clinical laboratory under normal conditions. 683 isolates were obtained from clinical specimens, sequenced, and analysed over the course of 30 months. The sequence provided species level identification for 568 of these isolates (83.1%). The identification was limited to the genus level for 108 isolates (15.8%), and the sequence remained unidentifiable by 16S rRNA sequence analysis for 7 isolates (1%). The 16S rRNA approach failed to identify bacteria to the taxonomic level for three reasons: 72 isolates (66%) failed to differentiate between species, 15 isolates (13.8%) lacked any closely related sequence in the database, and 13 isolates had more than 1% undetermined position in the sequence (12 percent) (S Mignard,J P Flandrois 2006).

2.5 Production of PHB

PHB is the most common biopolymer that can replace non-biodegradable polymer used in different sectors. Application of PHB in various sectors depends upon its biodegradability, biocompatibility, thermal and mechanical properties regulated by upstream and downstream processing used in fermentation. Here, we studied biodegradation of PHB produced by *Bacillus* sp (Dash Ankita et.al 2010).Polyhydroxyalkanoates (PHAs) can be synthesized by adopting different microbial fermentation strategies. Solid-state fermentation (SSF) could be a possible strategy for the cost-effective production of PHAs. The major drawback to address is the proper maintenance of the culture conditions under SSF (Raveendran Sindhu*et.al*2017).

2.7 Isolation of PHB

The isolation and purification of bacterial polyhydroxyalkanoates is a critical step in the fermentation system's profitability. As a result, many scientists have looked into this field in

order to develop this biodegradable polymer. The ideal method should result in a high level of purity and recovery at a low cost of production. This paper discusses four isolation methods: halosolvent and non-halosolvent extraction, non-polyhydroxyalkanoate cell material digestion using surfactants, sodium hypochlorite, or enzyme, mechanical cell disruption methods such as bead mills and high pressure homogenization, and new methods such as spontaneous liberation of poly(3-hydroxybutyrate), dissolved air flotation, air classification, or supercritical CO2. The pretreatment of cell disruption, as well as polyhydroxyalkanoate purification and analytical methods, are also discussed(Nicolas Jacquel*et.al* 2008).

2.8 Purification of PHB

The purification procedure for the isolate's PHB was carried out according to the published method as*Haloarculasp. AB19* cells containing intracellular polymer were extracted from 1L of culture broth by centrifugation at 10,000 rpm for 10 minutes, washed once with sterile water to lyse the cells, and then resuspended in water and lyophilized at -80°C. PHB was extracted from lyophilized cells in a Pyrex Soxhlet apparatus for 30 hours with hot chloroform and concentrated by evaporating the solvent under vacuum. The PHB was precipitated from the concentrated solution using 10 volumes of ethanol, and the resulting PHB granulates were filtered twice (Apexa H. Soni*et.al* 2012).

2.9 Isolation ofSoya protein

Graphene aqueous dispersion was employed to modify the renewable, compatibleand biodegradable soy-protein-isolated (SPI) films to enhance their thermal stability, mechanical stability and water resistance. Atomic force microscopy (AFM) images confirmed the monolayer structure of graphene (Yufei Han et.al 2017).Soy protein isolate and high-methoxyl pectin biopolymers were studied. Solubility, charges, turbidimetry, and optical microscopy analyses were conducted. Soy protein isolate showed low solubility at pH 4.5, but complete solubility at pH 11.0. The formation of complexes suggested an attractive interaction between the biopolymer **(**Tian Tian *et.al* 2019**).**

2.10 Study the biopolymer

The goal of this study was to characterize the soy protein isolate and high-methoxyl pectin biopolymers separately, as well as the complexes formed by both at various proportions

and pHs, in order to determine the best pH and biopolymer ratios for use as food stabilisers. Solubility, charges, turbidimetry, and optical microscopy were used to evaluate the biopolymers, while turbidimetry and optical microscopy were used to evaluate the systems with the pair of biopolymers. At all pH tested, high-methoxyl pectin had a high solubility. Solubility of the soy protein isolate was low at pH 4.5, which is close to its isoelectric point, and complete at pH 11.0. With high absorbance reading values and images of complexes from optical microscopy, the formation of complexes suggested an attractive interaction between the biopolymers. The goal of this study was to characterize the soy protein isolate and high-methoxyl pectin biopolymers separately, as well as the complexes formed by both, at various proportions and pH, in order to determine the best pH and pH range. Positive charges were present in systems with pH below the isoelectric point of the soy protein isolate; however, the high-methoxyl pectin had negative charges (Irian Luisa*et.al.* 2017)

3. Objectives of Research / Proposed Hypothesis:

3.1. Physico chemical analysis of collected marine water sample.

3.2 Screening of PHB producing halophilic bacteria from marine water.

3.3 Electron Microscopic study of the isolate

3.4 Culture dependent and culture independent analysis (16S rRNA sequencing) of the isolated bacterial species.

3.5 Insilico analysis of identified cultures through bioinformatics tools

3.6 Production of PHB by surface and submerged fermentation (using laboratory glass Bioreactor).

3.7 Statistical analysis of PHB production by the isolates.

3.8 Purification of PHB by various chromatographic methods.

3.9 To study Soya protein isolation and preparation of biopolymer

3.10 Synthesis of biopolymer by using PHB and Soya protein.

3.11 Analytical study of the formed biopolymer.

4. Methodology to be Adopted:

4.1 Study area:

Marine water (Juhu beach Mumbai, Maharashtra, India) will be used for isolation of halophilic microorganisms.

4.2 Sample collection:

The marine water sample will be collected in a sterile bottle to avoid contamination (Nidhi patel*et.al* 2017).

4.3 Physico-chemical analysis of sample:

Physico-chemical analysis of marine water will be analyzed using standard procedures prescribed in American Public Health Association (APHA) such as water temperature, Odor, Taste, TDS, COD, BOD, DO, pH etc.(Devangee shukla*et.al* 2013).

4.4 Screeningof PHB producing Halophilic bacteria:

Water samples will be taken from Mumbai marine water than the 5 mL of sample will be added to 100 mL of medium containing a salt concentration in between 15- 25% (Dunda's, Sea water agar, Halophilic Medium, Luria Bertani) for enrichment. Than the loopfull of sample streak or spread on the Dunda's, Sea water agar, Halophilic Medium,Luria Bertani (LB) agar these agar(Containing 15-25% salt) will be used for screening of PHB producing halophilic microorganisms. After the samples will be incubated for 7 days at 35±02°C(Nidhi patel*et.al* 2017, Sepideh Zununi Vahed*et.al* 2011).

4.5 Staining and Electron microscopic study:

The Sudan black B will be used to stain PHB of halophilic microorganisms. The isolated marine halophilic cultures will study by electron microscopy (Nidhi patel *et.al* 2017).

4.6Physico-chemical Characterization of PHB production from halophilic microorganisms:

Physico-chemical parameters will be used for the high production of PHB from isolated halophilic bacteria such as temperature, pH, agitation, carbon source, nitrogen source, metal ions etc. (Christina Thapa*et.al* 2018).

4.7 Identification of isolates by culture dependent and culture independent (16S rRNA sequencing) methods.

The marine bacterial species will be characterized by using biochemical tests. The isolated marine bacterial species will send to Agharkar Research Institute (ARI), Pune for 16S rRNA sequencing.

4.8 Bioinformatics:

Insilico analysis of identified cultures through bioinformatics tools for gene sequencing.

4.9 Production of PHBby surface and submerged fermentation process:

Surface and submerged fermentation processes will be used to produce PHB. Glass 0bioreactor will used for the PHB production. The PHB production medium containing 20 g/L glucose, 2 g/L yeast extract, 25g/L NaCl; 0.25 g/L $MgSO{\cdot}7H_2O$, 0.09 g/L $CaCl_2{\cdot}2H_2O$, 0.5 g/L KCl, 0.25 g/L KH_2PO_4, 0.06 g/L Na Brand Nile red 0.5 microgram dye per mL of medium or Dunda's medium or HM or SW or any other cheap raw material based medium will use for fermentation. The isolated cultures can incubate at 37°C for 10-15 days (Christina Thapa*et.al*2018).

4.10 Isolation of PHB:

After the fermentation is finished, the fermenting broth will collect and centrifuge at 4°C for 15 minutes at 10,000 rpm. A 4% sodium hypochlorite solution will be used to digest the pellet. The pellet will be collected by centrifugation at 10,000 rpm for 15 minutes, followed by washing and extraction with water, acetone, and ethanol. The polymer will have dissolved in chloroform and allowed to evaporate completely. The extracted dry PHB's will be calculated using following equation.

Residual biomass (g/ml) = DCW (g/ml) – Dry weight of extracted PHB (g/ml)

PHB accumulation (%) = Dry weight of extracted PHB (g/ml) × 100 / DCW (g/ml)

(Nabila Fathima S*et.al* 2017).

4.11Purification of PHB:

Chromatography is a technique used in the laboratory to separate a specific component from a mixture. For the separation of PBH from fermented broth, chromatography methods such as gel chromatography, ion exchange chromatography, affinity chromatography and other methods can be used (R. Z. Sayyed*et.al* 2020).

4.12.Extraction of soya protein:

Soybeans will peel, crush, and sift through a 100-mesh screen. Flours will degrease three times with n-hexane 1:6 (w/v) in a 37°C water bath. Defatted soy flours can be mixed with deionized water at a ratio of 1:10 (w/v) with the pH adjusted to 8.0 (2 mol/L NaOH). The mixture will have stirred continuously for 2 h in a 50°C water bath. Afterward, the solutions will centrifugeat 10,000 rpm for 30 min at 4°C. After centrifugation, the pH of supernatant will adjustto 4.5 using2 mol/L HCl, and centrifuge at 6500 rpm for 30 min at 4 °C. The

precipitate will wash with deionized water at 4°C and neutralized to pH 7.0 using 2 mol/L NaOH, followed by dialysis, and freeze-drying. The final product will be soya protein isolates(Tian Tian *et al*, 2019).

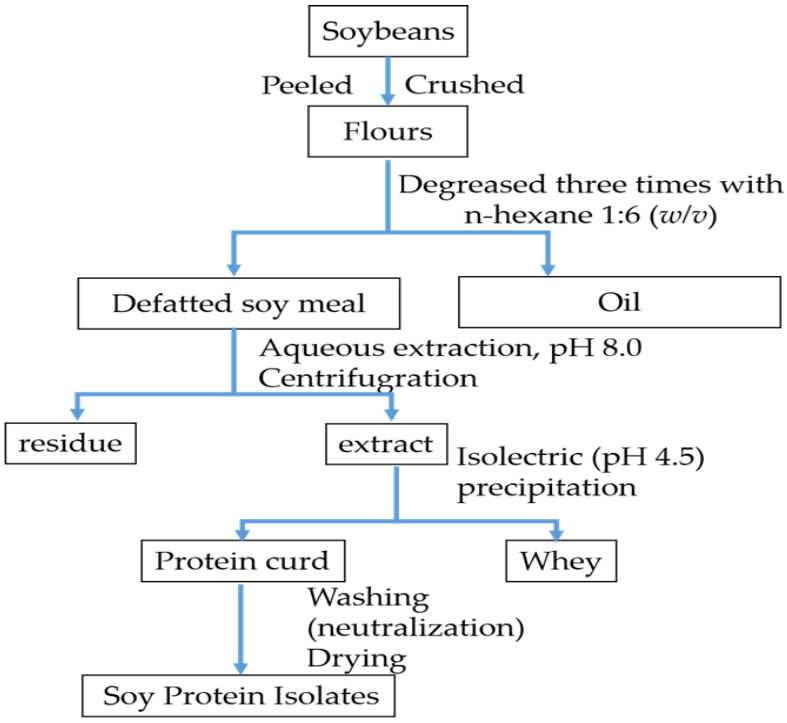

Fig: Soya protein extraction (Tian Tian*et al*,2019).

4.13.Synthesis of biopolymer using PHB and Soya protein:

Chemical modification of the partially purified PHB and soya protein may be used for biopolymer production. It strengthens the chemical and physical characteristics of the produced polymer as a result of the modification. The synthesized polymer may be characterized by using analytical methods.

5. Importance of study / society application:

Importance of study

Plastics are a useful resource for society, as they often provide functionality that other materials cannot simply or affordably replace. The majority of plastics is quite durable and can survive for hundreds of years. They've replaced metals in most manufactured goods component, including computers, vehicle parts, and refrigerators, and in the process, they've made them cheaper, lighter, safer, stronger, and easier to recycle. Plastics have mostly replaced paper, glass,

and cardboard in packaging, lowering costs and cutting carbon emissions while also better protecting the objects they protect. Bioplastics have a variety of (potential) advantages in the search for new material solutions while keeping in mind the goal of sustainable production and consumption. Bioplastics with controllable physical properties can now be made, allowing for applications that are similar to those of oil-based plastics. Some bioplastics have properties that enable them to perform new functions. Bioplastics can be processed in the same way that petrochemical plastics are, including injection moulding, extrusion, and thermoforming. Bioplastic polymers can be blended with co-polymers or other polymers to increase their tensile strength.

- The waters and coastlines are defiled by plastic trash. Plastic poisoning kills sea animals and fish. Since 1945, about 5% of the world's total plastic production has ended up in the oceans. Every country in the world has shopping bags and other packaging strewn across its streets and fields.
- The plastics sector consumes a lot of energy, which is usually derived from fossil fuels, and hence contributes to global greenhouse gas emissions.
- Due to the obvious endurance of plastics, we will continue to experience landfill strain unless we have effective and widespread recycling. Plastics are a very visible contributor to trash disposal concerns, while not being the largest category of materials entering landfills (that honour goes to building waste).
- Toxic chemicals are used extensively in the production of traditional polymers.
- Some polymers absorb minor amounts of chemicals into the environment, including endocrine disruptors. These substances have the potential to harm both animals and humans. (The remedy to this problem is to avoid employing original raw components - either monomers or plasticizers - that could result in such compounds when the plastic is in use or destroyed.)

Biopolymers made of PHB and biopolymers made of soya protein have no strengthening properties and are not resistant to high temperatures or strong acids. As a result, when soya protein is added to PHB, it undergoes a modification that may improves its straightness, temperature resistance, acid resistance, and other chemical and physical properties. The main goal of present investigation is to determine which legends and couplers are helpful to couple soya proteins to PHB

Society Application

1. **Biomedical:** Sutures, supports of tissue cultures for implants, surgical implants, dressings, part of bones and replanted veins, engineering of heart valves and pins.
2. **Pharmacological:** Encapsulation of medicines for controlled release
3. **Packaging:** Food packaging
4. **Veterinary:** Encapsulation of veterinary medicinal products
5. **Agricultural:** Encapsulation of fertilizers
6. **Environmental:** Bags, bottles, disposable items, items of personal hygiene, films of involvement, degradable diapers and remediation of areas affected by oil spills.
7. **Industrial:** Recovery of oligomers and monomers for new use in the synthesis of polymers.

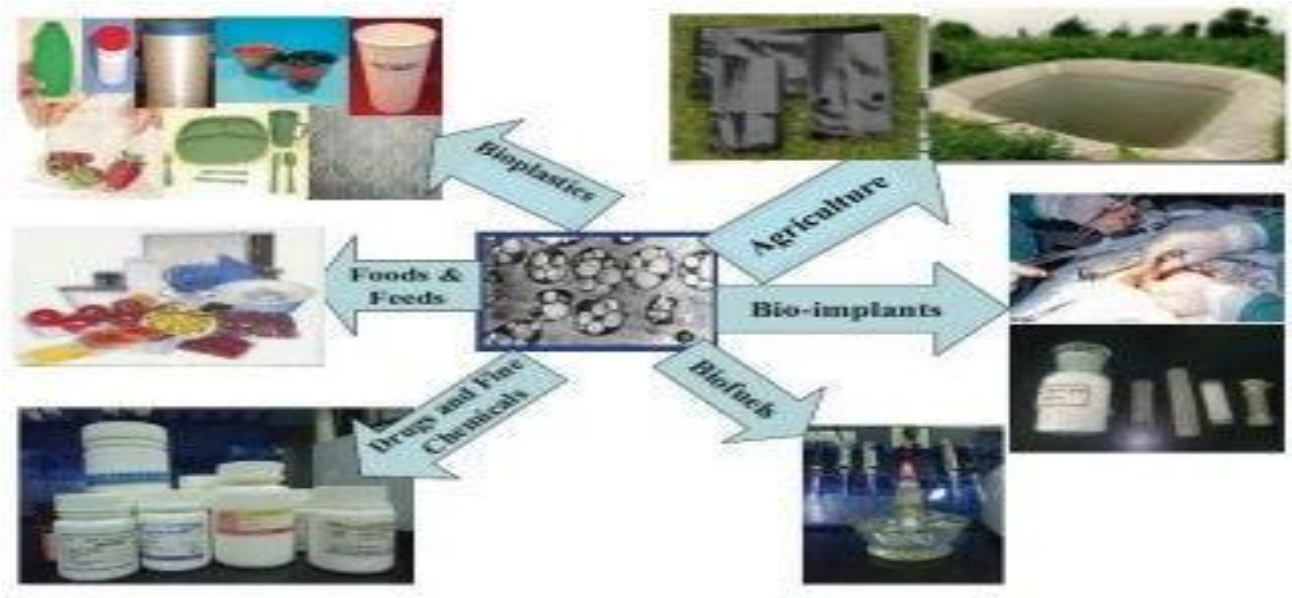

Fig: Application of PHB(Antonio José dos Santos *etal*.2017).

6. Year wise planning of Work

First Year

6.1 Reference/Literature Collection:

6.2 Collection of Sample

Sample will be collected from Mumbai juhu beach marine water.

6.3 Physico-chemical Characterization of water

The physico-chemical contents of marine water samples will be tested to determine pollution levels, pH, minerals, salts, and heavy metal concentrations.

6.4 Screening and Isolation of PHB producing Halophilic bacteria

Marine water sample containing Halophilic bacteria for these enrichment will be used Dunda's both, HM SW, Luria Bertani (LB) (with 7.5% NaCl) and screening will be done by using Dunda's, HM, SW, Luria Bertani (LB) agar.

6.5 Microscopic study of the isolates

PHB Producing granules will be visualized byelectron microscope.

6.6 Characterization of isolated strains

The isolated halophilic bacterial species will be characterized by Biochemical tests, statistical analysis, growth curve measurement, quantitative analysis and morphological analysis.

6.7 Identification of isolated Halophilic bacterial strain

The halophilic bacterial specie will identify by 16S rRNA sequencing method.

6.8 Bioinformaticanalysis of isolated Halophilic bacterial strain.

Insilico analysis of identified cultures (16S rRNA sequence) through bioinformatics tools.

6.9 Submission of Progress Report.

6.10 Preparation of Research Paper.

Second Year

6.11.Production of PHB

The surface and submerged fermentation methods will be used for high production of PHB from halophilic bacterial species. The glass bioreactor will be used for production of PHB.

6.12.Physico-chemical parameter used for the production of PHB.

Different type of parameter such as PH, Temp, substrate conc., % of inoculums, time period, effect of NaCl Conc., effect of carbon source etc. to check its effects on yield of PHB production.

6.13PHB isolation.

Fermented broth will be centrifuged at 10000 rpm for 15-20 min at 4°C for crude PHB separation. After centrifugation collect the supernatant for PHB purification and pellet will discard.

6.14Purification of isolated PHB.

The gel filtration, TLC, Affinity, ion exchange chromatography etc.such techniques may be applicable for purification of PHB.

6.15Statistical analysis of PHB production by the isolates.

One-way analysis of variance (ANOVA)used to determine statistical differences between different media for the same microorganism.

6.16.Isolation of Soya protein from soya beans

Soya bean will be taken and crush it for the isolation of soya protein. Flours will degrease using hexane and separating protein and oil. Proteins will be precipitate in its isoelectric point. Further its purification will use dialysis.

6.17.Submission of Progress Report

6.18Preparation of Research Paper

Third Year

6.19Preparation of biopolymer by using PHB and Soya protein.

The extracted partially purified PHB undergoes a chemical modification by adding soya protein and specific coupling ligands which may results, increases its straightness and modify its chemical and physical properties. During chemical modification the used arms, couplers or ligands will be decided after the finding of chemical structure of PBH and used soya protein.

6.20To study the biopolymer by analytical methods

Physical, chemical and biological properties of these biopolymer are checked out by using analytical methods such as; antibacterial activity, straightness, surface morphology, SEM(scanning electron microscopic) analysis etc.

6.21Preparation of Graphs

6.22 Data Interpretation

6.23 Final Compilation of Data

6.24Preparation of Research Paper & Communicating it for Publication

6.25Report Writing

6.26Submission of Final Report to University with all details.

7. References:

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